SIGNIFICANCE OF THE pH OF BLUEBERRY LEAVES[1]

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(WITH TWO FIGURES)

Ecologically, the swamp, or highbush blueberry, *Vaccinium corymbosum* L., differs fundamentally from other cultivated plants in its requirements for an acid soil, a constant source of moisture (**6**), and a relatively high anion nutrient uptake (**19**). In conjunction with studies on blueberry nutrition presented elsewhere (**19**), data were obtained on the effect of mineral nutrient deficiencies and age of leaves on the pH of the blueberry leaf sap. It was thought that these studies on the pH of the leaf sap might offer the key to an understanding of the mechanism by which the swamp blueberry can live in its usual environment.

Materials and methods

Dormant rooted blueberry cuttings of the Cabot variety were planted on January 7, 1942, in 54 coffee urn liners (glazed crocks) containing quartz sand, and accommodating two cuttings per crock. Beginning ten days later, nine different nutrient solutions were applied, designed to induce the following mineral nutrient deficiencies: None, nitrogen, phosphorus, potassium, calcium, magnesium, sulphur, iron, and boron. The composition of the solutions and the method of application have been previously described (**19**). As an added precaution, each solution was adjusted to a pH of 4.8–5.0 with hydrochloric acid. In all, six liners (12 plants) were treated with each of the nine solutions. Four plants from each treatment were removed for analyses on February 22 (46 days after planting), four others on March 15 (67 days after planting), and the remaining four on April 10, 1942 (93 days after planting).

Determinations of the pH of the plant sap were made by means of a Coleman pH electrometer (model 3). Immediately after the removal of each plant, the sap for pH determinations was prepared by grinding a representative part of the current leaf growth in a mortar with a few milliliters of distilled water and quartz sand and decanting the supernatant liquid into the cuvette of the pH apparatus.

The titration curves (fig. 1) were constructed from data obtained as follows: Two grams of fresh leaves of the respective plant species growing in the greenhouse were thoroughly macerated with 100 ml. of distilled water in a Waring blendor, transferred, and made up to 250 ml. with additional distilled water. A 50-ml. aliquot was removed, to which increasing quantities of N/15 hydrochloric acid were added, with subsequent measurement of the pH following each addition of the acid. Another 50-ml. aliquot was similarly treated with increasing additions of N/15 sodium hydroxide.

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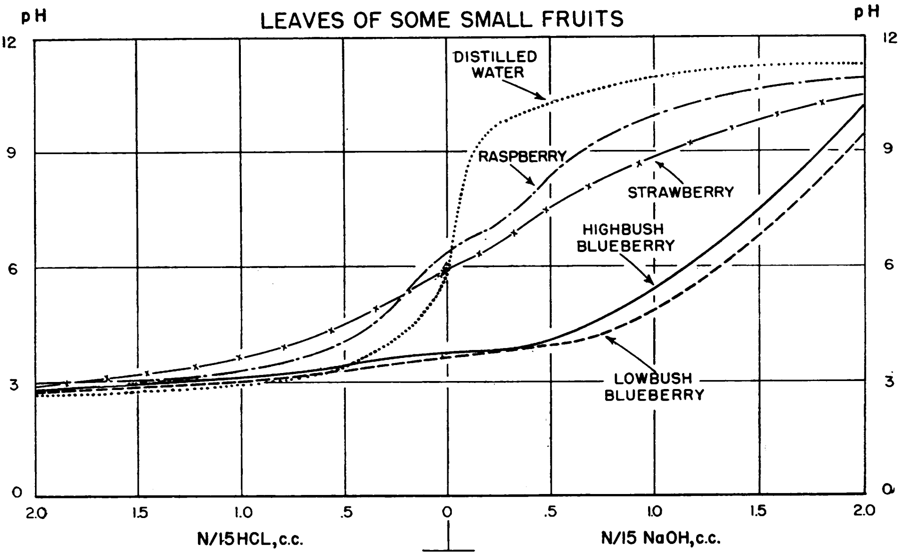

FIG. 1. Titration curves of blueberry leaves compared to other small fruits.

In order to separate the water-soluble constituents of the blueberry leaf tissue from the residual material (fig. 2), additional blueberry leaves were macerated, filtered, and made up to 250 ml. The residual material was dispersed for several minutes in additional 250 ml. of distilled water. Only 25-ml. aliquots were used for these titrations and consequently the titration curves thus obtained are not directly comparable to those presented in figure 1.

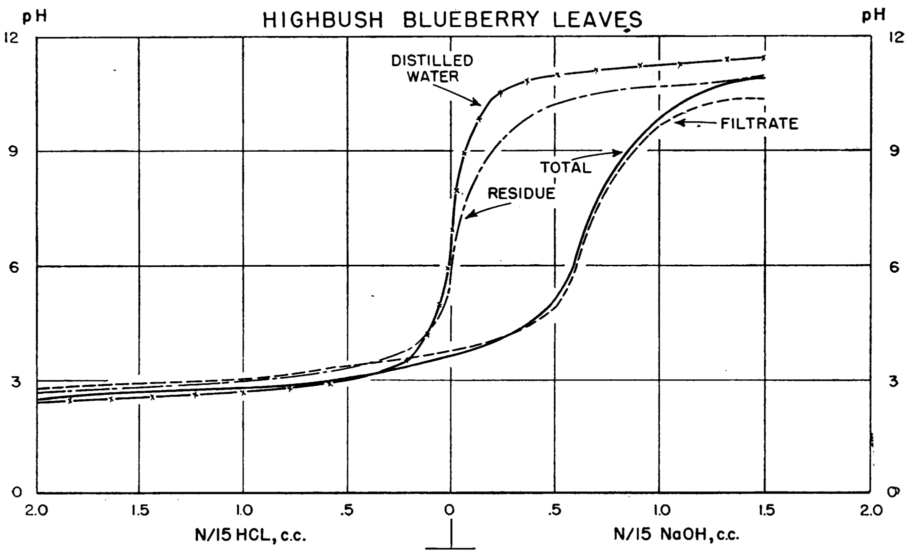

FIG. 2. Titration curves of the water soluble filtrate compared to the residual material of blueberry leaves.

An indication of the isoelectric point of the water-soluble proteins in the blueberry leaf tissue was obtained from a filtrate prepared in a similar manner from 20 grams of fresh leaves and made up to a volume of 500 ml., by adding 25-ml. aliquots to each of a series of beakers containing varying amounts of hydrochloric acid or sodium hydroxide. After the aliquot was added to each beaker the pH of its contents was determined; it was then filtered, the precipitate dried down to constant weight, and weighed. The pH value at which the greatest precipitation occurred was assumed to be the isoelectric point for water-soluble proteins. It is believed that the severe agitation of blueberry leaves during maceration caused the proteins to denature.

TABLE I

EFFECT OF AGE AND MINERAL NUTRIENT DEFICIENCIES ON THE pH OF BLUEBERRY LEAVES. FIGURES REPRESENT AVERAGES OF FOUR ROOTED CUTTINGS PLANTED IN SAND CULTURES ON JANUARY 7, 1942

DEFICIENCY	REMOVED ON FEB. 22, 1942		REMOVED ON MARCH 15, 1942			REMOVED ON APRIL 10, 1942	
	LEAVES	LEACHINGS	LEAVES		LEACHINGS	LEAVES	
			YOUNG	OLD		YOUNG	OLD
None	3.66	4.4	3.45	3.70	4.01	3.43	3.77
Nitrogen	3.69	6.0	3.56	3.67	5.93	3.44	3.67
Phosphorus	3.70	4.4	3.55	3.74	3.98	3.77	4.45
Potassium	3.67	4.2	3.75	3.96	4.02	3.59	4.31
Calcium	3.90	4.5	3.81	4.26	4.25	3.87	4.06
Magnesium	3.78	4.4	3.80	4.17	4.31	3.65	4.07
Sulphur	3.67	4.5	3.70	3.86	4.08	4.00	4.40
Boron	3.90	4.6	3.50	3.85	4.13	3.53	3.87
Iron	3.65	4.2	3.65	3.82	3.99	3.50	3.75
F value	8.37*	......	1.25	3.90*	1.46*	2.85*	3.37*
Significant difference	0.15	......	....	0.30	0.27	0.27	0.37

* Statistically significant at odds of 99 : 1.

Results

The younger blueberry leaves have a lower pH than the more mature leaves (table I). The difference in the pH of young and old leaves was least in the nitrogen-deficient plants.

When young leaves were sampled separately, no significant effect of mineral deficiencies on pH was found until the last removal (93 days after planting) when the young leaves deficient in phosphorus, calcium, or sulphur were higher in pH than the young full nutrient leaves. This may have been an indication that those plants ceased active growth, and thus even the leaves towards the tip of the shoot were older than they would have been had growth continued normally.

When old leaves were sampled separately, calcium- or magnesium-deficient leaves were significantly higher in pH by the second removal (67 days after planting) and with the exception of the minus-nitrogen and minus-iron plants, all of the older nutrient deficient leaves showed a higher pH than the old full nutrient leaves by April 10 (93 days after planting). In general, the leaf areas that were necrotic for any reason whatever were least acid, the pH of such areas reaching as high as 5.0.

The leachings from all of the pot cultures, with one exception, approximated the pH value of 4.4 at the first removal and 4.0 at the second removal. The pH leachings of the minus-nitrogen cultures, however, averaged close to pH 6.0.

The pH titration curves for leaf sap of blueberries and other small fruits are given in figures 1 and 2. It is obvious that not only is the hydrogen-ion concentration of blueberry leaf sap strikingly greater than that of the other plant species, but the titratable acidity of the blueberry is also much higher. The data presented in figure 2 indicate that practically all the acidifying substances are readily water soluble.

When aliquots of the water-soluble filtrate of blueberry leaf tissue were added to solutions of different pH's, the greatest precipitation occurred at pH 5.0, thus indicating that the isoelectric point of the water-soluble proteins in blueberry leaves is at approximately that point.

Discussion

The above results are significant when viewed in the light of the concepts developed by ROBERTSON (**21**), PEARSALL (**20**), and others, regarding the relation of the hydrogen-ion concentration of plant sap to the isoelectric point of the plant proteins. Briefly, they found that a double maximum of physico-chemical activity (*e.g.*, imbibition, swelling, etc.) exists when the plant sap pH is at either side of the pH of the isoelectric point of the plant proteins. This plant activity diminishes as the pH of the plant sap approaches the pH of the isoelectric point of the plant proteins. Actually, however, saps of all plants examined by them and later by others (**7, 9, 18**), had a decidedly higher pH than that of the isoelectric point of the respective plant proteins.

For the blueberry, however, the reverse seems to be true; in fact, the pH of the blueberry plant sap is two to three full points below that of most cultivated plants, as illustrated in figure 1. In other words, the hydrogen-ion concentration in blueberry leaves is 100 to 1000 times greater than in leaves of most cultivated plants. Whereas in many plants the younger and more vigorous parts are higher in pH than older plant parts (**7, 12, 17, 18, 21, 24**), in the blueberry the younger parts are lower. Conversely, whereas various deficiencies (**8, 9, 10, 11, 18, 22**) and incidences of disease (**13, 14, 16, 21, 23, 24**) caused a decrease in the pH of other plants, such conditions usually resulted in an increase in the pH of the blueberry plant sap. It appears, therefore, that in contrast to most plants, the pH of blueberry leaf sap *rises* towards the pH of the isoelectric point of its proteins upon senescence or the occurrence of disease or injury, since the isoelectric point of the soluble blueberry leaf proteins was found to be approximately at the pH of 5.0, while the pH of young, healthy leaves was about pH 3.5.

Since the leaf sap of the blueberry does appear to be well on the acid side of the isoelectric point of its soluble proteins, are not the following speculations justified? Is it not probable that the amphoteric proteins, in contrast to their behavior in most plants (**3, 20**), act in the blueberry as cations? Then if some of the plant proteins act as cations, would that not partially explain the preferential absorption of anions (**19**); and if anion radicals are absorbed in excess, would it not be difficult to keep iron in the blueberry plant in the reduced, available form, thus explaining the frequent reports of iron deficiency in blueberries (**2**)? Furthermore, in the presence of relatively large quantities of anion radicals which in themselves may serve as oxidizing agents (**1, 15**), would there not be less need for the oxidizing effect of the manganese ion, thus explaining the absence of a beneficial effect on blueberry growth from the application of manganese (**19**)?

From the practical standpoint it is interesting that the pH value of the minus-nitrogen plants remained low, and there was little difference in pH between the younger and older minus-nitrogen leaves; pH might therefore serve as a diagnostic tool for confirming suspected nitrogen deficiency in blueberries.

In an attempt to combine into one basic concept some of the results of blueberry plants when compared with results obtained with other plants, especially in the light of some recent findings on the mechanism of mineral ion intake and the significance of the hydrogen ion concentration of plants, the following generalization was postulated: The behavior of the swamp blueberry may be based upon its capacity to thrive under soil conditions limiting aeration, but supplying at the same time adequate moisture to meet rather exacting water requirements of the plant. Experiments supporting this view indicate that the blueberry has exacting moisture and oxygen requirements, high anion and low cation uptake, a requirement for highly acid soils, an extremely acid plant sap, and that the pH of its plant sap is on the acid side of the isoelectric point of its soluble proteins.

Considering the natural occurrence of the blueberry on soils with high water tables, how can the experimental evidence be used in explanation of a special adaptation of the blueberry to such conditions? Because of the high moisture requirement blueberries usually thrive on soils with high water tables. Lack of aeration in such soils is overcome not by the presence of aerenchyma tissue, but by the extremely shallow root system and a high soil acidity where oxidation rather than reduction processes prevail (**5**). Since such soils are low in calcium and other exchangeable bases, a low cation requirement is necessary for plants to exist in such an environment, while the satisfaction of the high anion requirement may increase the usually limited supply of oxygen needed for respiration and organic acid formation. The high organic acid content is responsible for the low pH value of the plant sap, which is below the isoelectric point of the "soluble" plant proteins. These proteins may consequently act as cations, thus balancing the presence of excessive anions. The prevailing shade of the natural environment aids in further maintaining the low pH by preventing organic acid destruction (**4**).

When the blueberry is transplanted from its natural habitat into ordinary cultivated soils, it probably suffers from lack of moisture, and since it may ordinarily obtain a part of the oxygen used in respiratory processes from anion radicals, suffers from lack of such nutrients. The destructive effect of high light intensities on the organic acid content of the plant and the consequent increase in pH towards the isoelectric point may also be a factor of measurable effect on the growth of blueberries in cultivated fields where no shade is provided.

In conclusion, it seems reasonable to raise the following question: From a physiological standpoint, is the blueberry (and perhaps similar plants, the cranberry for example) radically different from most cultivated plants, and if it is different, should not comprehensive physiological studies of such plants be undertaken in order to arrive at a broader understanding of plant processes in general?

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